

b.) Amendments to the Specifications

The phrases “The amounts of polypeptides in the protein standard represent the equivalent amounts of BSA used in the quantity estimation.” are added in Examples 1-3 after the protein estimation for explanation of the meaning as directed by the examiner. The entire paragraphs in Examples 1, 2 and 3 of the Specification are repeated as directed by the examiner.

EXAMPLE 1

Prepare chicken egg white from a fresh commercial chicken egg. Dissolve the chicken egg white proteins in water by vortex. Estimate the total protein mass by BioRad protein assay (BioRad, Hercules, CA) and by Coomassie Blue staining of a SDS gel containing chicken egg white proteins with bovine serum albumin (BSA) as a standard. The amounts of polypeptides in the protein standard represent the equivalent amounts of BSA used in the quantity estimation. Prepare the dissolved chicken egg white proteins at concentration of 1 milligram per milliliter in 50 mM Tris HCL pH 8.0, 1 mM EDTA, 1% SDS, 1 mM DTT. A protein standard from chicken egg white is made. Load 10 microliter of the protein standard on 12% SDS polyacrylamide gel. Electrophorese the gel at a constant current of 40 mA per gel. Stain the gel for 10 minutes with Coomassie blue staining solution (0.25% Coomassie brilliant blue R-250, 10% acidic acid, 45% methanol). Destain the gel with 7.5% methanol and 5% acidic acid overnight (above 16 hours). Three major protein bands will be visible on the gel under this condition. They are conalbumin, ovalbumin, and lysozyme. Their sizes are about 80, 43, and 14 kD respectively. Their masses are about 2, 7.5, and 0.2 micrograms (ug) respectively. See Fig. 1.

EXAMPLE 2

Estimate the concentration of each of these polypeptides by BioRad protein assay and by Coomassie Blue staining of a SDS gel containing these polypeptides and different masses

of BSA as a standard. The amounts of polypeptides in the protein standard represent the equivalent amounts of BSA used in the quantity estimation. Mix these recombinant polypeptides RP, GA, BT, XL, and TR at concentrations of 100, 50, 30, 20, and 10 microgram per milliliter respectively in 50 mM Tris HCL pH 8.0, 1 mM EDTA, 1% SDS, 1 mM DTT. A protein standard with recombinant polypeptides is made. Load 10 microliter of the protein standard on a precast 4 to 20% gradient gel (Norvex, San Diego, California). Electrophorese the gel at a constant current of 40 mA per gel. Stain the gel for 10 minutes with Coomassie blue staining solution. Destain the gel with 7.5% methanol and 5% acidic acid overnight (above 16 hours). Five major protein bands will be visible on the gel under this condition. They are RP, GA, BT, XL, and TR. Their sizes are about 100, 55, 40, 30, and 20 kD respectively. Their masses are about 1, 0.5, 0.3, 0.2, and 0.1 micrograms (ug) respectively. See Fig. 2.

EXAMPLE 3

Prepare BSA, lysozyme and aprotinin (Roche Molecular Biochemicals, Indianapolis, IN) at concentration of 10 miligram per mililiter. Estimate the concentration of each of these polypeptides by BioRad protein assay and by Coomassie Blue staining of a SDS gel containing these proteins with BSA as a standard. The amounts of polypeptides in the protein standard represent the equivalent amounts of BSA used in the quantity estimation. Mix the following polypeptides RP, BSA,GA, BT, XL, TR, lysozyme, and aprotinin at concentration of 10, 20, 50, 100, 300, 1000, 100, and 10 microgram per milliliter respectively in 50 mM Tris HCL pH 8.0, 1 mM EDTA, 1% SDS, 1 mM DTT. A protein standard with commercial natural and recombinant polypeptides is made. Load 10 microliter of the protein standard on a precast 4 to 20% gradient gel (Norvex, San Diego, California). Electrophorese the gel at a constant current of 40 mA per gel. Stain the gel for 10 minutes with Coomassie blue staining solution. Destain the gel with 7.5% methanol and 5% acidic acid overnight (above 16 hours). Eight major protein bands will be visible on the gel under this condition. They are RP, BSA,GA, BT, XL, TR, lysozyme, and aprotinin. Their sizes are about 100, 66, 55, 40, 30, 20, 14, and 6 kD respectively.

Their masses are about 0.1, 0.2, 0.5, 1, 3, 10, 1, and 0.1 micrograms (ug) respectively.
See Fig. 3.